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The Hedgehog Hold on Homeostasis

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http://dx.doi.org/10.1016/j.stem.2015.10.010

The adult lung is largely quiescent, with airway epithelia turning over slowly. Peng et al. (2015) describe a key role for the Hedgehog pathway in actively maintaining this quiescence, a surprising turn of events given the pathway's established mitogenic role, and they show that Hedgehog pathway attenuation is required for proliferative regeneration.

Unlike rapidly regenerating tissues such as the intestine or skin, adult airways turn over slowly, on the order of several months. The lung therefore has emerged as a compelling "model organ" for investigating mechanisms of cell fate and homeostasis in long-lived adult tissue. Perhaps not surprisingly, signaling pathways central to embryonic development have also emerged as key players in the adult. These pathways, however, can play unexpected roles in maintaining homeostasis and restoring it after damage. Recently, in a reversal of the mitogenic paradigm typically ascribed to Hedgehog (Hh) signaling during development and cancer, Peng et al. (2015) report that Hh actively restrains the proliferative activity of adult lung mesenchymal cells to maintain quiescence. Furthermore, the Hh signal provides a key conduit through which epithelial and mesenchymal cells communicate, and attenuating this signal is required after damage to enable cell division, regeneration, and restoration of homeostasis.

Hh represents a highly conserved pathway that regulates numerous aspects of embryonic development, acting as a morphogen to regulate cell fate decisions in some contexts and as a mitogen to promote cell proliferation in others (Varjosalo and Taipale, 2008). In the developing murine lung, Sonic Hedgehog (Shh), one of three Hh ligands, is dynamically expressed in the nascent epithelia, while Patched (Ptch), which receives the Hh signal, and Gli1, an Hh effector, are Hh target genes that mark active Hh signaling in neighboring mesenchymal cells (Bellusci et al., 1997). Genetic manipulation of Shh levels yields dramatic lung phenotypes consistent with Hh driving mesenchymal proliferation (Bellusci et al., 1997; Litingtung et al., 1998; reviewed in Kugler et al., 2015).

Importantly, Peng et al. now extend the analysis of Shh signaling to the adult lung. They first establish, similar to the paracrine mode of action previously described during embryogenesis, that Shh is expressed in the lung epithelia, predominantly in the secretory club cells, and the signal is received in the adjacent mesenchyme. Cell marker analyses point to fibroblasts as the predominant Hh-active mesenchymal cells, and these cells appear quiescent over 12 weeks under homeostatic conditions.

Peng et al. then manipulate Hh signaling using a suite of genetic tools, with provocative results. In surprising contrast to the mitogenic activity described in other contexts, perturbing Shh signaling increased mesenchymal proliferation in the adult. This increase followed Hh disruption at both ends of the paracrine signal: following deletion of Shh in the signal-sending epithelial cells and deletion of Smoothened (Smo), the Hh effector, in the signal-receiving mesenchymal cells. Conditional Smo deletion in lineage-traced Gli1 mesenchymal cells increased proliferation and expanded this population, providing the authors' strongest support for a cell-autonomous role of Hh signaling in the adult mesenchyme.

Injury models, based on chemical damage to the epithelium, yielded additional insights. Correlating with loss of the Shh-producing club cells, the Hh signal appeared lower (albeit modestly) in the mesenchyme following chemical insult of the epithelium. Lineage tracing from Gli1-expressing mesenchymal cells demonstrated that epithelial injury induced expansion of this population, possibly in a clonal fashion. Genetically forced activation of Hh signaling in these mesenchymal cells, using an oncogenic form of Smo, attenuated this expansion. Conversely, suppressing Hh signaling through Smo deletion in these same mesenchymal cells prevented restoration of homeostasis, which otherwise was recovered in wild-type controls over several months. Thus, Hh not only regulates a mesenchymal response to epithelial damage, but it also functions to restore mesenchymal quiescence following damage and repair.

To complete the loop of epithelial-mesenchymal communication, Peng et al. then addressed whether perturbations of Hh signaling in the mesenchyme might alter proliferation in the epithelial layer, under both homeostatic and regenerative conditions. The authors particularly focused on epithelial club cells, given that they not only provide Shh but also function as a "not undifferentiated" adult progenitor important for epithelial homeostasis and regeneration (Rawlins et al., 2009). Constitutive deletion of Shh from club cells or Smo from mesenchymal cells correlated with increased proliferation in bronchial club cells, although induced deletion of Smo caused only a trend (not statistically significant) toward increased proliferation. Following 2 months of recovery after epithelia injury, forced Hh activation in Gli-expressing mesenchymal cells resulted in a marked loss of secretory cells, whereas Hh inactivation generated a complementary result, namely excessive secretory cell proliferation and bronchial hyperplasia. In a final set of ex vivo experiments, Peng et al. showed that adult lung mesenchyme is required to support the growth of epithelial organoids

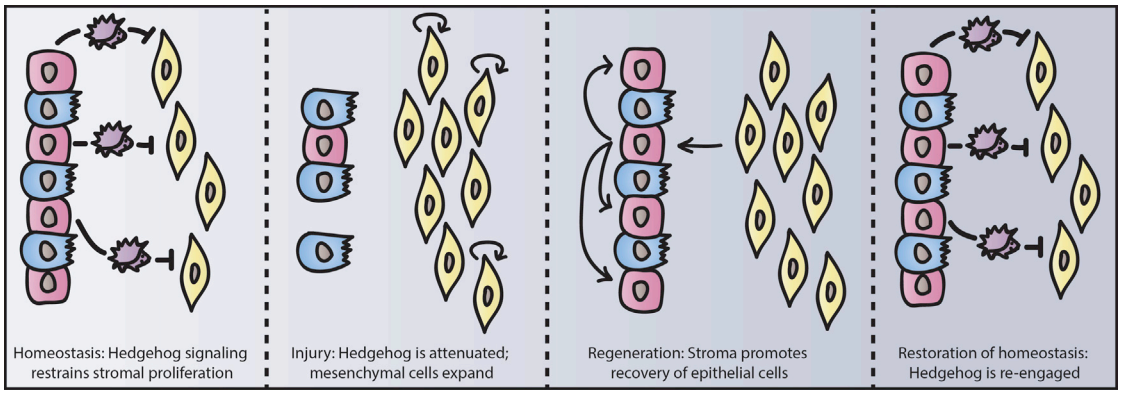

Figure 1. Hedgehog Signaling Sets Up an Epithelial-Mesenchymal Feedback Loop to Maintain Quiescence in the Adult Lung
Shh, expressed primarily in the secretory cells of the airway epithelium, drives Hh pathway activity in the adjacent mesenchyme and maintains its quiescence. Epithelial injury attenuates the signal, which relieves the quiescent hold on the fibroblasts, enabling them to proliferate. Mesenchymal expansion is in turn associated with increased proliferation of the epithelial layer, reestablishing the source of Hh ligand and a return to quiescence.

expressing markers of the secretory lineage. In contrast, Hh activation in the co-cultured mesenchyme reduced both the number and size of these epithelial colonies. The authors thus conclude that Hh signaling in the mesenchyme indirectly promotes epithelial quiescence.

These data lead to an intriguing model in which Hh signaling not only does not promote proliferation, but actively maintains quiescence in the adult lung (Figure 1). On the one end of the paracrine Hh signal, the airway epithelium secretes Shh to maintain mesenchymal quiescence. On the other end, the mesenchyme completes a feedback loop that helps maintain epithelial quiescence. Following damage, when homeostasis is perturbed and a reparative proliferative program must be engaged, damage-induced disruption of this Hh feedback loop relieves the Hh hold on quiescence. Regeneration of the epithelial layer, particularly the club cells, is then proposed to restore normal Shh production, which in turn reestablishes the hold on homeostasis. This model nicely fits with well-established ideas of paracrine signaling in multiple organ systems. On the other hand, it clearly deviates from earlier models that highlight a mitogenic role of Hh signaling in the adult mesenchyme. For example, Hh signaling has been proposed to contribute to the pathological expansion of the fibroblast population in idiopathic pulmonary fibrosis, a candidate therapeutic indication for pharmacological inhibitors of the pathway (Bolaños et al., 2012; Hu et al., 2015; Moshai et al., 2014). One superficially similar precedent to the Peng et al. model might be the deletion of Indian Hedgehog from the adult intestinal epithelia, which results in a wound-healing-like response characterized by an influx of fibroblasts and increased epithelial proliferation (van Dop et al., 2010). However, a number of experiments in the Peng et al. paper support a different mechanism, with a cell-autonomous and direct role for Hh signaling in the mesenchyme. A provocative new study such as this one of course raises questions. Does acute inhibition of Hh signaling, for example through pharmacological means, yield similar results? Does Hh function as a proliferative brake in other cell types? What factors does the Hh signal regulate in the mesenchyme to feed back to the epithelium? Do manipulations of other pathways that alter cell fate and decrease club cell numbers (and thus a source of Shh) affect mesenchymal proliferation and perturb homeostasis? Given the rapid progress that has recently emerged from signaling studies of the adult lung, it seems safe to assume that addressing such questions in this model organ will continue to yield important insights into how a slowly turned over adult tissue maintains homeostasis and responds to damage.

ACKNOWLEDGMENTS

The authors are employees of Genentech, Inc., a member of the Roche family.

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